THE LARVAL DEVELOPMENT AND METAMORPHOSIS OF THE ASCIDIANS *PYURA PRAEPUTIALIS* (HELLER) AND *PYURA PACHYDERMATINA* (HERDMAN) (PLEUROGONA, FAMILY PYURIDAE)

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Synopsis

Pyura praeputialis and *P. pachydermatina* are self-fertile hermaphrodites. In the Sydney region, artificial fertilisation (self or cross) yields larvae of *P. praeputialis* in April, May and September, and of *P. pachydermatina* in May, June and July. Larvae cannot be obtained by this method at other times of the year.

The egg of *P. praeputialis* is 0·23 mm in diameter, that of *P. pachydermatina*, 0·25 mm. At 20–23°C, the tadpole larvae of both species hatch within 12 hours of fertilisation. The trunk of the larva in both species is relatively large and yolky. In *P. pachydermatina*, the anterior adhesive papillae are long and prominent.

Tadpoles of both species begin to settle and metamorphose within 1–2 hours of hatching. Settlement occurs readily on glass. Degenerative changes in the larval tail begin at the distal end. Muscular contractions break up the tail structures in *P. pachydermatina* but not in *P. praeputialis*.

Ampullae develop on the zooid after settlement. In *P. praeputialis*, the 8 ampullae function in attachment and as a support for the tunic. The 4 ampullae of *P. pachydermatina* do not function in attachment. The attachment stalk of *P. pachydermatina* develops in the region of the adhesive papillae.

Siphons are developed in both species within 20 hours of settlement. By this time the zooid has begun to differentiate the pharynx, intestine and other adult structures, though subsistence on yolk continues for several days.

In comparison with other pyurids, although the eggs of *P. praeputialis* and *P. pachydermatina* are among the larger eggs in the family, embryonic development is rapid and the free-swimming larval stage is brief. The rudiments of the permanent organs show no differentiation until after settlement.

Introduction

Larval development and metamorphosis have been described for several species of Pyuridae (*Boltenia echinata*, *B. hirsuta*, Berrill, 1929, 1948 ; *Pyura squamulosa*, *P. microcosmus* Millar, 1951, 1954 ; *Herdmania pallida*, Sebastian, 1953 ; *Halocynthia pyriformis*, Berrill, 1929, 1935 ; *Halocynthia roretzi*, Hirai, 1941, 1968 ; see also Berrill, 1950, 1955). All of these species are oviparous and fall within the range of ascidian species categorised by Berrill (1935) as having small eggs, 0·26 mm or less in diameter, with a basal yolk/cytoplasm ratio and an unspecialised mode of development.

The pyurid fauna of the New South Wales coast includes two large species in abundance, *Pyura praeputialis* (Heller) and *Pyura pachydermatina* (Herdman). The mode of development of these species has not hitherto been known. *P. praeputialis* has generally been referred to in Australia as *P. stolonifera*, e.g. by Dakin (1952), Kott (1952) and Endean (1955), but has more recently been separated by Millar (1963) from the closely related *P. stolonifera* of South Africa. Millar's ruling, accepted by Goddard (1972), has been followed by the present authors.

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Goddard (1972) investigated the seasonal changes in gonad weight relative to body weight in *P. praeputialis* and determined probable breeding seasons. The seasonal cycle of *P. pachydermatina* has not been investigated. In the present work, Goddard's results on the seasonal cycle of *P. praeputialis* are confirmed, the seasonal cycle of *P. pachydermatina* is established and the development of both species is described.

Materials and Methods

Specimens of *Pyura praeputialis* and *P. pachydermatina* were collected at monthly intervals from February 1973 to January 1974. All of the specimens used in this study were obtained from intertidal rock platforms in the Sydney area, either on the open coast (Cape Banks at the entrance to Botany Bay) or in the lower reaches of Port Jackson (Dobroyd Point and Balmoral on the northern shore ; Bottle and Glass Rocks on the southern shore). *P. praeputialis* is common on the lower littoral zone at these localities. *P. pachydermatina* is found at the lower littoral margin, accessible on spring low tides. No specimens of the estuarine form of *P. praeputialis* were examined in the present study.

In the laboratory, each animal was removed from its test and opened to expose the gonads. The state of maturity of the gonads was assessed by visual inspection and was then tested by attempting artificial fertilisation. Eggs were released from the gonad by separating off portions of ovarian tissue and slicing the tissue finely in filtered sea water. The eggs were then teased free, washed in four changes of filtered sea water and dispensed into glass Petri dishes in sufficient numbers to provide an even, slightly spaced distribution on the bottom of the dish. A sperm suspension was obtained by slicing portions of testis in a Petri dish of filtered sea water. Approximately 2 ml of sperm suspension was added to each dish of eggs. After 30 minutes, the water was changed to remove excess sperm. In dishes in which development ensued, the water was changed at three-hourly intervals to prevent the infestation of the embryo cultures by protozoans. All cultures were maintained at 20–23°C.

At each attempted artificial fertilisation, both self-fertilisation and cross-fertilisation were tested for individuals of each species. When ripe gametes were available, both methods yielded larvae. No differences in development were observed between embryos obtained by self-fertilisation and those obtained by cross-fertilisation. Natural spawning was not observed in the laboratory for either species.

When development was obtained, embryos and larvae were removed from the culture dishes at intervals and fixed in 5% formalin-seawater for subsequent examination. Representative fixed specimens were embedded in paraffin (M.P. 56°C), sectioned at 7 μm and stained with Harris' haematoxylin and eosin.

Results

Seasonal Cycles

In this investigation, the occurrence of normal development following artificial fertilisation was taken as evidence that the animals contained mature eggs and sperm and were potentially capable of spawning. The question of whether spawning in the field is coincident with these times, or has a more restricted occurrence, was not investigated.

Pyura praeputialis : Using this criterion, the gonads of many individuals of *P. praeputialis* were found to contain mature eggs and sperm during the late autumn months, April and May. At this time, the ovarian portions of the gonads were distended with olive green eggs and the testicular portions were swollen and white. Many of the eggs released by dissection had a layer of follicle cells attached to the external surface of the chorion (Fig. 1A), and sperm

suspensions showed a high level of activity. Artificial fertilisation yielded embryonic development followed by the hatching of larvae which settled and underwent metamorphosis.

In June, July and August, the winter months, the gonads and gametes of *P. praeputialis* retained the same appearance as in April and May, but the results of artificial fertilisation were different. The June and July tests yielded some early development of the eggs, many reaching the gastrula stage, but development usually became arrested at this stage. A few eggs completed embryonic development, but the tadpoles that hatched were abnormal and died soon after hatching. The August tests produced a few cases of early cleavage only, with no development beyond this stage.

In the September samples, some specimens of *P. praeputialis* showed a reversal of this condition, with normal embryos and larvae resulting from artificial fertilisation. By October, however, only a few abortive larvae could be obtained, and from November to March, no development was observed in any of the monthly tests using artificial fertilization. In November, December and January, the gonads were small and lacked the distinctive coloration of ripe gonad tissue. This coloration, olive green in the ovarian portions and white in

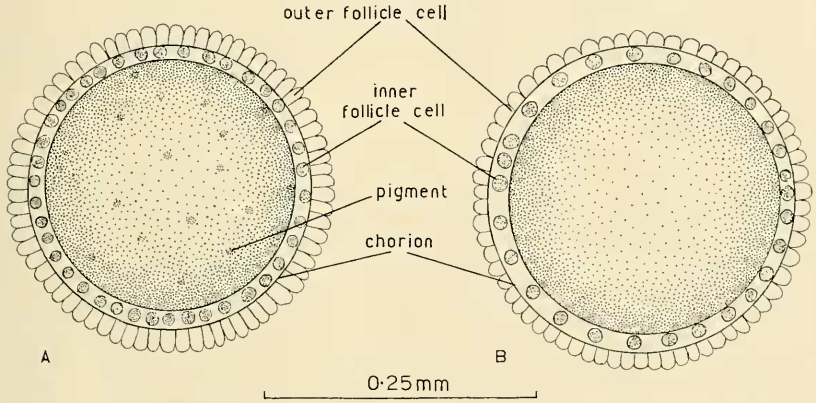

Fig. 1. Unfertilised eggs. A. *Pyura praeputialis*. B. *Pyura pachydermatina*.

the testicular portions, reappeared in some individuals in February and March, but the gametes extracted during these months showed signs of immaturity. The eggs had no follicle cells attached to the chorion, and the sperm, although active, had vacuoles attached to their tails. Artificial fertilisation tests using these gametes yielded no more than a few abortive early cleavage divisions.

In summary, therefore, the occurrence of mature gametes capable of yielding normal development after artificial fertilisation is confined in *P. praeputialis* to the months of April and May, with a minor resurgence in September.

Pyura pachydermatina : The seasonal changes in the gonads and gametes of *P. pachydermatina* are more sharply defined than those of *P. praeputialis*. During February and March and also in April immature gametes were plentiful but no eggs of mature size were found in the gonads of any individual examined. Sperm were obtained from some individuals during these months, but were not fully active and had vacuoles attached to their tails. In May, June and July, in contrast, the gonads of all individuals in each sample were distended, ovarian portions being olive green and testicular portions white. Artificial fertilisation resulted in embryonic development, normal hatching, settlement and metamorphosis.

During August, September and October, the gonads in *P. pachydermatina* remained superficially unchanged, but artificial fertilisation yielded only abortive embryonic development, with no hatching. Active resorption of gametes was evident in the November sample and, by December, none of the individuals examined had large gonads. Proliferation of immature gametes in the gonads was resumed in January.

P. pachydermatina in the vicinity of Sydney, therefore, has mature gametes in intertidal individuals of the population only in the late autumn to winter months, May, June and July.

Development

Gametes

The mature eggs of both species are spherical and yolky, with an external chorion and a layer of small follicle cells. Both are olive green in general coloration, but the egg of *P. praeputialis* is 0·23 mm in diameter and has small, reddish pigment spots scattered over its surface, while the egg of *P. pachydermatina* is 0·25 mm in diameter and lacks surface pigment spots. The chorion of an egg freshly removed from the ovary is attached to the egg surface, but lifts off after immersion of the egg in seawater, leaving a perivitelline space 30 μm wide. Inner follicle cells are visible in this space.

The sperm of *P. praeputialis* is about 50 μm long, with a rod-shaped head 7·5 μm long. That of *P. pachydermatina* is about 40 μm long, with a rod-shaped 5·0 μm head.

Embryonic Development

Development of the embryo follows the same general timetable in *P. praeputialis* and in *P. pachydermatina*. The first two cleavage divisions are completed within one hour after fertilisation and the next four divisions follow during the second hour. Cleavage is total, equal and radial (Fig. 2). Histological sections of cleaving eggs reveal a yolk-free cytoplasmic halo around each nucleus and a uniform distribution of yolk throughout the remainder of each cell. One pole of the 2-cell stage contains a cluster of large, refractile granules. By the 4-cell stage, the distribution of these granules has changed. In *P. praeputialis* the granules are now grouped around the nucleus in all four cells. In *P. pachydermatina* they are grouped around the nucleus in two cells only. The same pattern of peripheral yolk and central nucleated cytoplasm with associated granules persists in the 8-cell and later stages of cleavage, but the significance of the refractile granules is not known.

The hemispherical gastrula stage is attained in both species within 3 hours of fertilisation (Figs 2D, 4A). The ectoderm cells are still large and yolky, but most of the yolk is now located in the large endoderm cells lining the archenteron. With the completion of gastrulation, the archenteron is obliterated.

The tail rudiment becomes visible by 5–6 hours (Fig. 3) and completes its elongation to encircle the body of the embryo by 8–10 hours. The yolk reserves now lie mainly in the mass of endoderm cells in the trunk rudiment. The ectoderm cells are smaller than in the gastrula, but still contain some yolk granules (Fig. 4B). The notochord cells are also yolky, but the large muscle cells of the tail are now yolkless and basophilic. The otolith and ocellus are visible in the trunk by this time and the anterior adhesive papillae are beginning to develop.

The Tadpole Larva

In embryo cultures maintained under continuous illumination, hatching in both species begins about 12 hours after fertilisation. The tadpole larvae of the two species are easily distinguished (Fig. 5).

The larva of *P. praeputialis* (Fig. 5A) is 1·0–1·1 mm long. The trunk is 0·30 mm long and 0·24 mm deep, with three short, slightly tapered papillae at the anterior end. The tail is 0·70–0·80 mm long. The larva of *P. pachydermatina*

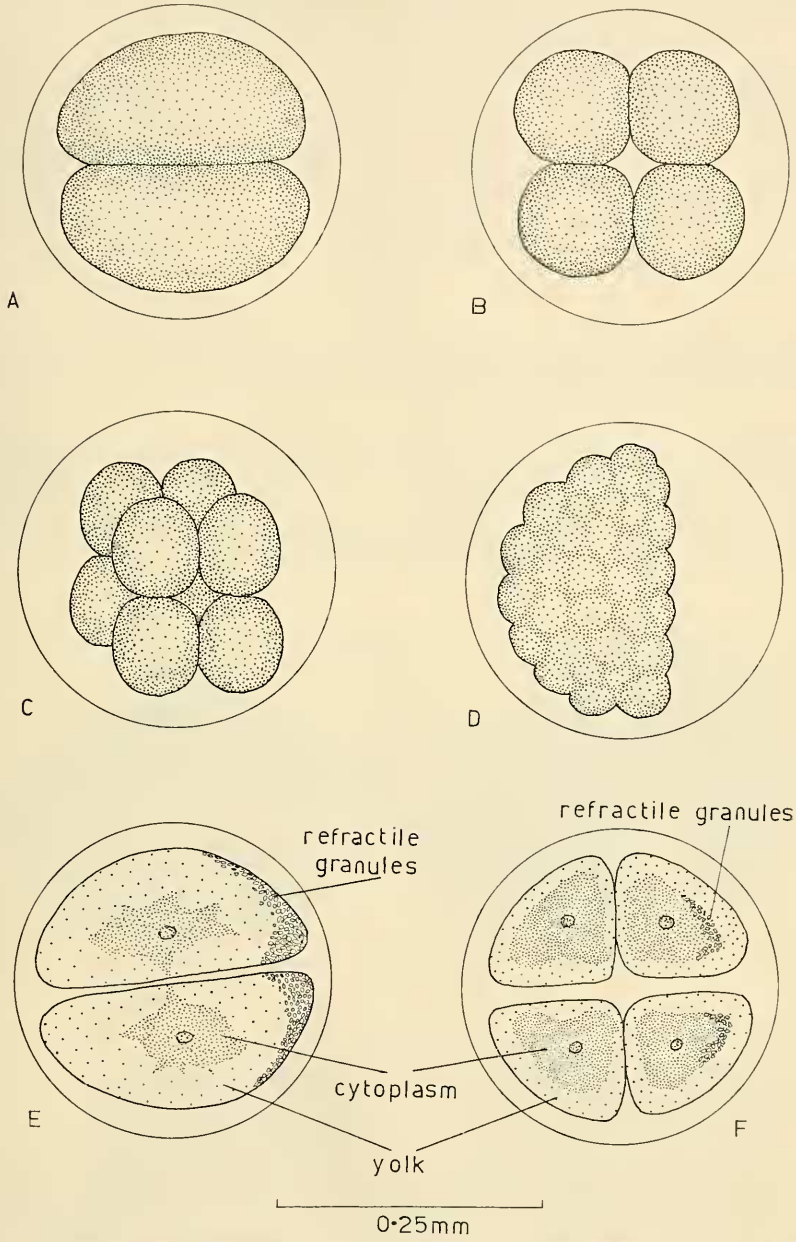

Fig. 2. *Pyura pachydermatina*. A. 2-cell stage. B. 4-cell stage. C. 8-cell stage. D. gastrula. E. 2-cell stage in section. F. 4-cell stage in section.

(Fig. 5B) is 0·9–1·0 mm long, with a trunk 0·30 mm long and 0·17 mm deep. The anterior papillae are more prominent than in *P. praeputialis*, being longer, thicker and set more widely apart. The tail is 0·60–0·70 mm long. The dorsal

and ventral tail fins of *P. praeputialis* are larger, more regularly shaped and more distinctly striated than those of *P. pachydermatina*, but no differences in swimming activity were observed between the two species. Both tadpoles swim about randomly in short bursts and are negatively phototropic.

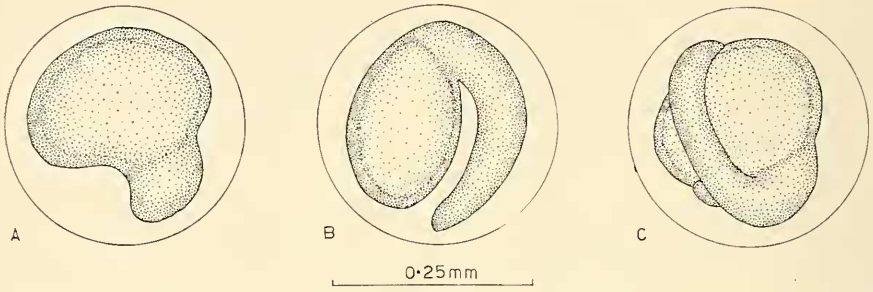

Fig. 3. *Pyura pachydermatina*, stages in development of the tail. A. 5–6 hours. B. 7–8 hours. C. 8–10 hours.

Histological examination (Fig. 6) shows that the ectoderm cells of the larval trunk, though small, still contain some yolk. The anterior papillae are protrusions of ectoderm, with a few mesoderm cells in the interior. The ectodermal cells of the papillae are enlarged and glandular. The dorsal ectoderm shows no sign of the invagination of the rudiment of the peribranchial sacs.

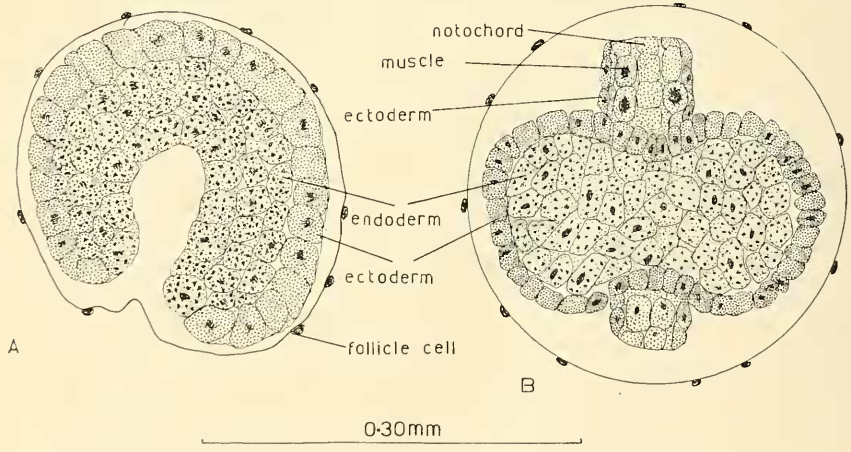

Fig. 4. *Pyura praeputialis*. A. gastrula in section. B. 8–10 hour embryo in transverse section.

Inside the trunk, the endoderm remains a mass of yolky cells, with no development of a central lumen or epithelial arrangement. The cerebral vesicle with its otolith and ocellus is embedded dorsally in the endodermal mass. Posteroventral to the endoderm is a compact mass of small, darkly staining mesoderm cells, clustered around the anterior end of the notochord.

In the tail, the larval muscle cells are similar in both species but the notochord of each is distinctive. In *P. praeputialis*, the anterior face of each notochord cell is concave, leaving a disc-shaped vacuole between successive cells. The nucleus

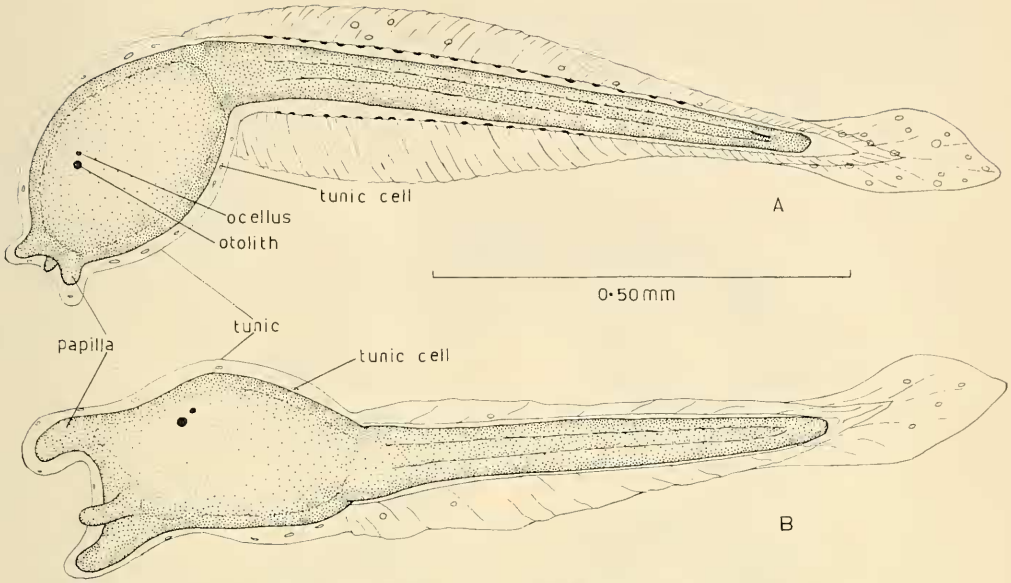

Fig. 5. A. larva of *Pyura praeputialis*. B. larva of *Pyura pachydermatina*.

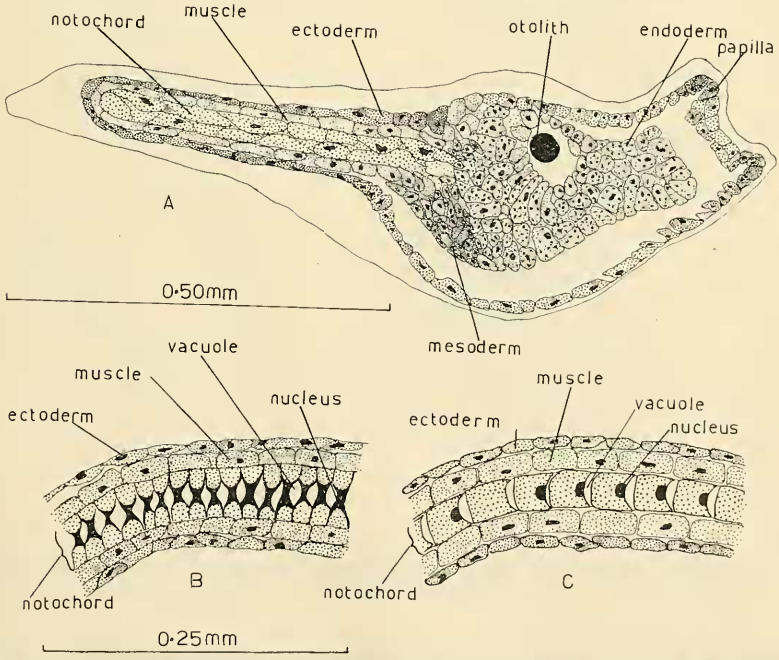

Fig. 6. A. longitudinal section of the larva of *Pyura pachydermatina*. B. frontal section of the larval tail of *Pyura pachydermatina*. C. frontal section of the larval tail of *Pyura praeputialis*.

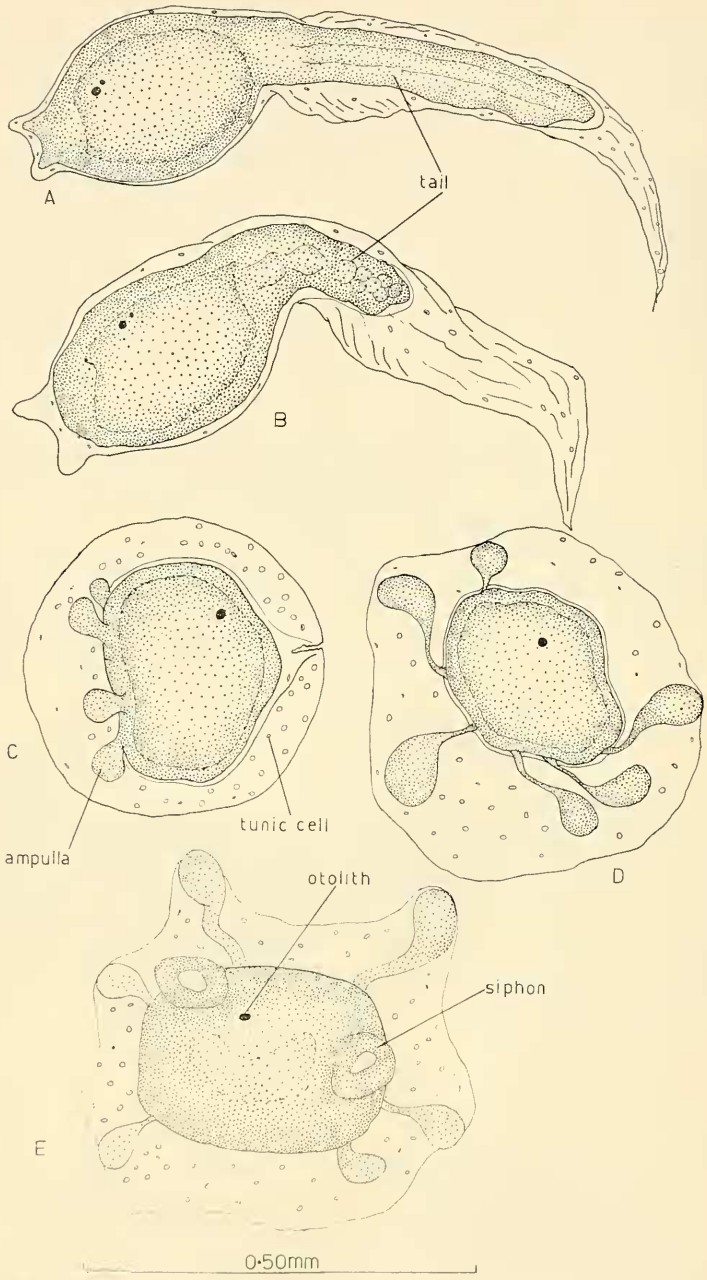

Fig. 7. Metamorphosis of *Pyura praeputialis*. A–B. stages in tail resorption. C–D. development of ampullae. E. development of siphons.

is ovoid and lies at the anterior end of the cell (Fig. 6c). The notochord cells of *P. pachydermatina* are concave on both faces, leaving lens shaped vacuoles between the cells (Fig. 6b). The nucleus occupies the narrow central region of the cell and is irregular in form.

Settlement

Within 2 hours of hatching, the tadpole in both species has settled and begun to metamorphose. Attachment to the substratum occurs in the usual manner by the anterior papillae and swimming activity then ceases. Following attach-

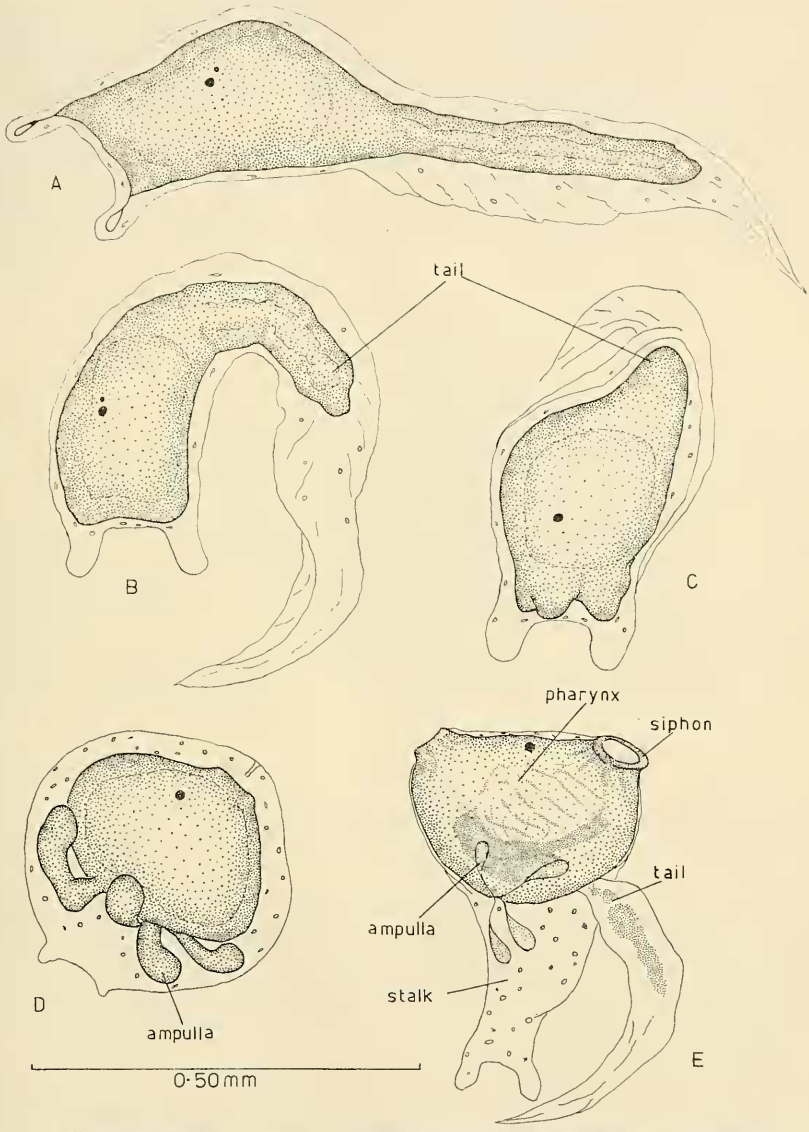

Fig. 8. Metamorphosis of *Pyura pachydermatina*. A–B. stages in tail resorption. C–D. development of ampullae. E. development of stalk and siphons.

ment, the papillae withdraw from the tunic, leaving the latter attached to the substratum. The tadpoles settled readily on the glass of their containers. Metamorphosis also began in tadpoles which did not become attached to the substratum, but was not completed in these individuals.

Metamorphosis

The first external change in the metamorphosis of both species is reduction of the tail (Figs 7 and 8). In *P. praeputialis*, this change is accompanied by expansion of the space between the trunk and tunic. The resorption of the tail tissues begins at the tip, where the notochord and muscle cells round up and become vacuolated. In *P. pachydermatina*, muscular contractions play a part in breaking up the tail tissues and pulling them into the trunk. These contractions do not occur in *P. praeputialis*. The tunic of the tail remains intact during resorption of the tail tissues and often persists into post-larval stages. The tail is fully resorbed 4–6 hours after settlement.

As the tail is resorbed, the trunk becomes more spherical. *P. praeputialis* develops a ring of eight ampullae arising from a central point. The ampullae extend into the space between the trunk and tunic, becoming long and thin. Some of the ampullae grow down towards the substratum and appear to have an attachment function. Others project upwards and may have a role in supporting

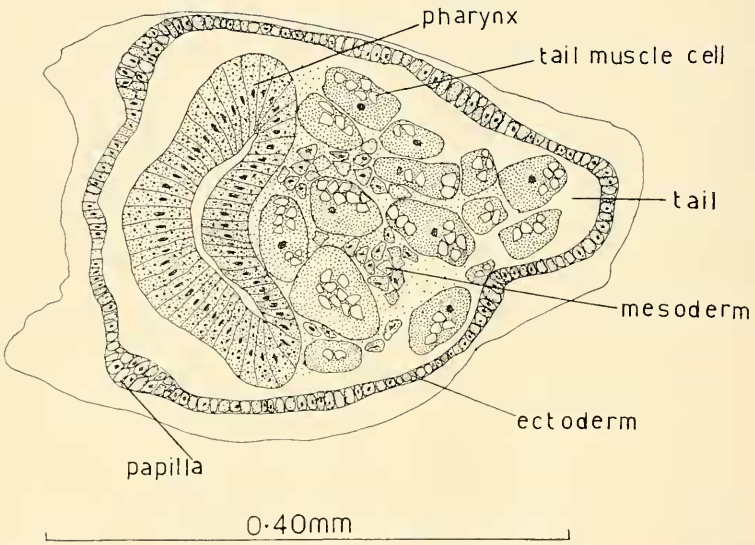

Fig. 9. *Pyura praeputialis*. Longitudinal section through a stage intermediate between Figs 7B and 7C.

the tunic. *P. pachydermatina* develops four ampullae, which do not have an attachment function. Initial attachment in this species is followed by extension of the tunic at the site of attachment, to form the stalk. The ampullae are folded back against the surface of the trunk during their early growth and do not project into the stalk region. They become thin and vessel-like in later settled stages with functional siphons (Fig. 8E).

The ampullae develop as ectodermal projections, but do not contain mesoderm cells. The endoderm is still a mass of yolky cells in the early settled stages of both species, but then becomes organised as an epithelium around a central lumen (Fig. 9). The vacuolated, degenerating tail cells can be seen in the body cavity of postlarval stages, surrounded by small cells derived from the mesoderm of the larval trunk. The otolith and ocellus remain visible in early settled stages, but the ocellus disappears as the ampullae become well developed.

The zooid in both species has developed oral and atrial apertures 15–20 hours after settlement. *P. praeputialis* shows regular contractions of the siphonal musculature 2 days after settlement. The otolith remains visible between the

siphons. Contractions of the siphons in *P. pachydermatina* are first seen approximately 4 days after settlement, by which time contractions of the heart and ciliary action in the pharynx are also visible.

The timing of development of *P. praeputialis* and *P. pachydermatina* at 20–23°C can be summarised as follows :

First and second cleavage divisions	$\frac{1}{2}$–1 hour
Later cleavage divisions	1–2 hours
Gastrula	3 hours
Tail rudiment forming	5–6 hours
Tail fully developed	8–10 hours
Hatching	12 hours
Settlement	13–14 hours
Tail resorbed	18–19 hours
Ampullae developing	20–24 hours
Oral and atrial apertures present	28–34 hours
Siphons contracting (*P. praeputialis*)	$2\frac{1}{2}$ days
Siphons contracting (*P. pachydermatina*)	$4\frac{1}{2}$ days

Discussion

Seasonality

The results obtained by Goddard (1972) indicated that *Pyura praeputialis* has a major breeding season in the autumn and a minor breeding season in the spring. The present study has confirmed this finding. *P. pachydermatina*, in contrast, has a single breeding season in the late autumn to early winter.

Among other pyurids, a similar restricted breeding season has been demonstrated by Sugawara *et al.* (1968) for *Halocynthia roretzi* in Japanese waters. Breeding in this species commences in the winter months, December to January, and continues for about two months. Several pyurid species of the northern hemisphere are known to breed in the summer (*Boltenia echinata*, *B. hirsuta*, Berrill, 1929, 1948 ; *Pyura squamulosa*, *P. microcosmus*, Millar, 1951, 1954 ; *Herdmania pallida*, Sebastian, 1953 ; *Halocynthia pyriformis*, Berrill, 1929, 1935) but the duration of the breeding season has not been established.

Embryonic and Larval Development

The egg diameter, the duration of embryonic development and the duration of larval swimming are now known for a number of species of pyurid ascidians (Table 1). Table 1 shows that the egg diameters of *Pyura praeputialis* and *P. pachydermatina* fall towards the upper end of the range of the family. It might therefore be expected, following Berrill (1935), that the duration of embryonic development and the length of free-swimming larval life in these species would be in general similar to those of *Halocynthia roretzi* and *H. pyriformis*, with eggs of similar diameter. Clearly this expectation is not fulfilled by the results of the present work. Even taking into account the temperature differences involved, *Pyura praeputialis* and *P. pachydermatina* develop to hatching and attain settlement with unusual rapidity. *Herdmania pallida* is the only species among those so far studied that has a similar rapid development, but the results quoted for this species (Sebastian, 1953) were obtained at exceptionally high temperatures and still include a longer free-swimming period than in *P. praeputialis* and *P. pachydermatina*.

An explanation of how *P. praeputialis* and *P. pachydermatina* attain settlement and the onset of metamorphosis with such rapidity can be suggested in terms of the mode of development of their larval and permanent organ rudiment. It has long been known (Berrill, 1931, 1935, 1955) that these two sets of rudiments in an ascidian embryo show considerable independence in development. In all

the pyurids that have been investigated, apart from *P. praeputialis* and *P. pachydermatina*, the rudiments of the permanent organs at hatching have already undergone some organogenesis and include a pharyngeal sac, an intestinal rudiment and sometimes a peribranchial invagination. Some further development of these rudiments takes place during the free-swimming period, and is then followed by the functional differentiation of the permanent organs after settlement has been attained.

In *P. praeputialis* and *P. pachydermatina*, however, the rudiments of the permanent organs do not reach this level of development in the embryo or larva. The endoderm remains a mass of yolky cells without a lumen. The mesoderm is a simple cluster of cells. The ectoderm cells retain yolk granules and there is no peribranchial invagination. In the period between the end of gastrulation and the completion of settlement, including the brief larval period, the rudiments of the permanent organs undergo almost no development. This brief time is devoted entirely to the development and functioning of the larval organisation. The further development and functional differentiation of the permanent organisation is delayed in these species until after settlement.

Table 1

Egg diameters and development in Pyuridae

Species	Egg diameter (mm)	Hatching at (hrs)	Free swimming (hrs)	Temperature (°C)	Reference
Pyura squamulosa	0·16	22	10	18–20	Millar, 1951
Boltenia echinata	0·18	34	10	16	Berrill, 1929, 1935, 1948
Boltenia hirsuta	0·18	34	10	16	
Herdmania pallida	0·19	8	3	26–29	Sebastian, 1953
Pyura microcosmus	0·20	24	24	18–20	Millar, 1954
Pyura praeputialis	0·23	12	1–2	20–23	this paper
Pyura pachydermatina	0·25	12	1–2	20–23	
Halocynthia roretzi	0·25	46	24	13–14	Hirai, 1941, 1968
Halocynthia pyriformis	0·26	60	—	16	Berrill, 1935

A simple change in the developmental relationship between the larval and permanent organisations therefore accounts for the brevity of embryonic development and larval life in *P. praeputialis* and *P. pachydermatina*. It does not, of course, explain why this brevity should have evolved in the two species. There may be advantages in a shortened duration of vulnerable free stages in the life cycle of an oviparous sessile species, but much more will have to be known about their population biology before this question can be answered.

Metamorphosis

In spite of the lesser degree of development of the permanent organs at settlement, the metamorphosis of *Pyura praeputialis* and *P. pachydermatina* also proceeds at a faster rate than those described for other Pyuridae, e.g. *Pyura squamulosa* and *P. microcosmus* (Millar, 1951, 1954). Resorption of the larval tail and development of the ampullae is completed within 12 hours of settlement in *P. praeputialis* and *P. pachydermatina* at 20–23°C, in contrast to 1–2 days in

the two British species at 18–20°C. The development of functional siphons within $2\frac{1}{2}$ days of settlement, as compared with 6–9 days, and the onset of feeding at 4 days, as compared with 12 days, are further indications of a generally faster rate of development after settlement in *P. praeputialis* and *P. pachydermatina* as compared with *P. squamulosa* and *P. microcosmus*.

The general pattern of metamorphosis and development of the permanent organs described by Millar (1951, 1954) for *Pyura squamulosa* and *P. microcosmus* is retained in *P. praeputialis*. The resorption of the tail and rounding up of the trunk are accompanied by the development of a ring of eight ampullae which serve temporarily in facilitating attachment of the test to the substratum. The ampullae are still prominent when the siphons become functional, and the otolith remains visible between the siphons. Metamorphosis in *P. pachydermatina* differs in several details. Only four ampullae are formed and they do not play a direct part in the growth of the attachment stalk of this species. By the time that the siphons are prominent, the ampullae in *P. pachydermatina* have become vestigial. The development of four ampullae rather than eight is a feature of *Boltenia echinata* and *Halocynthia pyriformis* (Berrill, 1929, 1948). A second feature of metamorphosis which *Pyura pachydermatina* shares with *Boltenia villosa*, but which does not occur in *Pyura praeputialis*, is the muscular contractile activity that plays a part in resorption of the larval tail (Barrington, 1968).

Acknowledgement

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